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Request for grant of a patent

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1. Your reference
5297601/HJF

2. Patent Application Number ~~25 AUG 1999~~ **9920170.9**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Patents ADP number *(if known)*

If the applicant is a corporate body, give the Country:
country/state of its incorporation State:

4. Title of the invention
A PASSIVE SAMPLING DEVICE

5. Name of agent **Beresford & Co**

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Patents ADP number 1826001

6. Priority details

Country	Priority application number	Date of filing

Patents Form 1/77

7. If this application is divided or otherwise derived from an earlier UK application give details

Number of earlier of application | Date of filing

8. Is a statement of inventorship and or right to grant of a patent required in support of this request?

YES

9. Enter the number of sheets for any of the following items you are filing with this form.

Continuation sheets of this form	
Description	66 ~~884~~
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Priority documents	
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Statement of inventorship and right to grant of a patent *(Patents form 7/77)*	1 ✓
Request for preliminary examination and search *(Patents Form 9/77)*	
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11. I/We request the grant of a patent on the basis of this application

Signature Beresford & Co BERESFORD & Co Date 25 August 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

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A PASSIVE SAMPLING DEVICE

The present invention relates to a device for the passive sampling of aquatic environments, its construction and use thereof in 'continuously' monitoring micropollutants in aquatic environments. In particular, this invention relates to a device comprising a receiving phase for the micropollutants that is separated from the aquatic environment by a diffusion-limiting membrane.

The monitoring of environmental waters for the presence of toxic inorganic (*eg* heavy metal) and organic micropollutants is a requirement of both domestic and European legislation. The purpose of this monitoring programme is two-fold: both for the assessment of long-term trends in pollutant levels and as a means of recording short-term or episodic periods of increased analyte concentration ("pollution events"). In either case, a technique is required for representatively sampling the aquatic environment that accounts for fluctuations of analyte concentration over time. Considerable spatial and temporal variations are known to exist at particular sampling sites, and the infrequent sampling of aqueous aliquots can lead to significant inaccuracies in the estimation of time-averaged concentrations. In addition, the monitoring of sometimes

very low levels of pollutants in the environment is technically very difficult using current technology.

In order to assess the long-term trends in pollutant levels, therefore, a technique is required for the 'continuous' and quantitative isolation of target analytes from the aquatic environment. This would not only allow for the accurate assessment of time-averaged concentrations to be made but would also provide a means of detecting the short-term elevations in levels found in pollution events.

Hitherto, the technique most commonly used for the monitoring of micropollutants in the aquatic environment is a programme of direct sampling of aqueous media. Possible pollutants are identified according to regional usage patterns, and representative samples are taken from specific sampling points at specific time intervals and are transported to an analytical laboratory for pre-concentration and instrumental analysis. For example, for the sampling of metal species, solid-phase extraction on C_{18} bonded silica cartridges or disks by filtration has been used, as described by Björklund and Morrison in Analytica Chimica Acta 343 259-66 (1997). This technique allows for the accurate determination of

specific compounds in a particular place and at a particular time. The results from these sampling events are correlated and used for the assessment of long-term trends in pollutant levels. However, in order to extrapolate these data successfully and thus to determine the true time-integrated pollutant levels accurately, samples must be taken with sufficient frequency that fluctuations in concentrations around the mean level can be accurately measured. Sampling programmes, therefore, are time-consuming, expensive and labour-intensive, and they have drawbacks in situations where large spatial or temporal variations in pollutant levels are observed. In addition, maintaining sample integrity during storage of samples prior to analysis is also problematic. Alterations in the sample over extended storage periods, even at low temperature, can result in an apparent reduction in analyte concentration.

To try to overcome the problems of sampling, another technique, known as biomonitoring, is also used. Biomonitoring is based on the ability of aquatic organisms to accumulate pollutants in their body tissues. Hydrophobic pollutants, in particular, can accumulate to levels that far exceed those found in the surrounding aquatic environment. This process is both active (by

means of ingestion of contaminated material) and passive (by means of diffusion of compounds from the aqueous environment through membranes of the body or gills into the lipid tissues of their bodies).

Biomonitoring studies make use of this process to monitor low-level contamination in aquatic environments. Aquatic organisms, usually bivalve molluscs, are exposed at specific sampling sites and the extent of accumulation of pollutant compounds in their body tissues is measured. Compounds in the organism become concentrated to levels far exceeding those found in the aquatic environment, which allows for ng/l levels of contaminants to be determined without the need for very large sample volumes to be analysed. As biomonitoring is an accumulative process, it effectively provides a means for the continuous, *in-situ* sampling of lipophilic organic analytes and metal species throughout the period of deployment, thereby providing information on the fluctuating analyte concentrations to which the organisms have been exposed.

However, the drawbacks of using aquatic organisms for monitoring levels of micropollutants include: The handling of tissue samples is time-consuming and may

require extensive clean-up prior to analysis. The choice of organism used for biomonitoring surveys is important; a single biomonitoring species will be restricted to a particular climate and water type, and all species are unsuitable for deployment in highly polluted sites where the survival rate of the test organisms is low. As bioconcentration is basically a partitioning process between the aqueous environment and the lipid tissues of the organism used, variations in percentage fat in each individual will alter the rate of accumulation. Rates of bio-accumulation also vary widely between individuals of different species and are also significantly affected by age, sex and general health of the test organism. In addition to the large inter- and intra-species variation in accumulation rates, some organic materials can be metabolised by certain organisms (*e.g.* many polyaromatic hydrocarbons are metabolised rapidly in the tissues of fish but not in the tissues of bivalve molluscs).

Attempts have therefore been made to develop techniques that exhibit the advantages of biomonitoring but without the above-mentioned disadvantages. Therefore, wholly passive (*i.e.* not requiring added energy) sampling devices have been developed in an attempt to provide an integrative technique for continuously monitoring aqueous

contaminant levels without the drawbacks of using living organisms or expensive electrically-powered equipment. Current systems comprise a liquid receiving phase having a high affinity for micropollutants that is separated from the aquatic environment by a porous membrane. They rely on the partitioning of pollutant species from the aqueous phase to the receiving phase by means of diffusion through the membrane.

The possible advantages of such systems are two-fold: First, they provide a means of continuously monitoring the levels of pollutants in the aquatic environment, thus providing information on fluctuating analyte concentrations. Secondly, pollutants are accumulated in the receiving phase to levels exceeding those in the environment, thereby enabling very low levels of contaminants to be determined.

By controlling the dimensions and materials used in the design of a particular device, the kinetics of uptake of micropollutants species within the sampler may be controlled. This partitioning behaviour has been related to the physico-chemical parameters of particular compounds. It may be possible by calibrating a test set of compounds in laboratory experiments to develop a model

which can be used to predict the uptake kinetics of an uncalibrated analyte based on its known physico-chemical parameters. This would render time-consuming calibrations for each pollutant unnecessary.

Various devices have been proposed for use in a passive sampling system. For example, GB patent specification no. 1 566 253 discloses a device comprising a glass vessel containing a non-polar solvent separated from the aquatic environment by means of a porous membrane. This device relies on the passive partitioning of freely-dissolved organic micropollutants from the aqueous environment through the porous membrane, such as cellulose, into a hydrophobic receiving phase, such as *n*-hexane.

However, the majority of known passive samplers for organic compounds make use of a liquid organic receiving phase held within a bag composed of flexible dialysis or polymeric membrane materials. For example, a development of the glass device, where the cellulose dialysis membrane is in the form of a bag filled with *n*-hexane, is described by Södergren in Environ. Sci. Technol. 21 855-859 (1987). This system has been used by a number of researchers for the accumulation of lipophilic pollutants

in laboratory and field trials.

An alternative design based on a non-porous polymeric enclosure or envelope ('bag') is disclosed in US patent specification number 5 098 573, and comprises a bag made from, for example, polyethylene filled with high molecular mass organics, such as the artificial lipid triolein (1,2,3-tri[*cis*-9-octadecenoyl] glycerol). This system, again, relies on passive diffusion of organic pollutants through, for example, a diffusion-limiting low-density polyethylene membrane and accumulation in the triolein reservoir. The rate of diffusion of non-polar organic species through low-density polythene material has been said to mimic the diffusion of the same species through biological membranes. The aim of this design compared to that of the *n*-hexane-filled dialysis bag, therefore, is more closely to imitate the passive uptake of organic pollutants in aquatic organisms. However, a drawback of this system is that analytes need to be extracted from the triolein and concentrated, prior to analysis.

Another approach has been proposed by Frantz and Hardy in J. Environ. Sci. Health, A33(7) 1275-90 (1998), for the determination of halogenated ether pollutants using time-

weighted-average concentrations of the pollutants. Their sampler comprised a glass tube having at one end a silicone polycarbonate membrane affixed with silicone cement and sealed at the other end with an aluminium foil-covered rubber stopper. Tenax (registered trademark), a porous polymer based on 2,6-diphenyl-p-phenylene oxide, was placed inside the sampler, which was then exposed to a stirred solution of the pollutants in an exposure tank having an aluminium lid for holding the sampler. However, such a device could not be expected to operate satisfactorily under field conditions in which, *inter alia*, the air in the glass tube would render the sample buoyant and likely to invert under turbulent conditions, thereby creating a substantial diffusion gap between the polycarbonate membrane and the Tenax; in any event, the Tenax would be capable of substantial movement. Furthermore, analysis thereof is rendered difficult by having to draw off the polluted extractant after the desorption step; and sealing of the polycarbonate membrane to the body of the sampler (the glass tube) also renders this impractical for repeated washing and re-use.

Therefore, despite various improvements resulting from these passive sampling devices, there are still important

deficiencies that require to be remedied. They are fragile and depend on the accumulation of analytes in a mobile receiving phase. This mobile receiving phase, in many designs, is prone to leaching from the system, making them unsuitable for deployment in environmentally sensitive areas. Some systems have inadequate protection against biofouling of the membrane surface. The biofouling of the membrane alters the uptake kinetics of the system rendering laboratory calibrations invalid. In some cases, it has been found that the uptake kinetics of the system are affected by turbulence. Other problems relate to ease of use, analysis and handling.

Accordingly, the present invention provides a device for continuously monitoring, during a deployment period ranging from a few hours through days to several weeks, micropollutants in an aquatic environment, which device comprises:

(a) a diffusion-limiting membrane capable of being in contact with the aqueous environment when the device is in use and adapted to allow rate-limited diffusion therethrough of the micropollutants; and,

(b) separated from the aqueous environment by the membrane, a receiving phase having a sufficiently high affinity for the micropollutants for continuously, during the deployment period, receiving and retaining the micropollutants

characterised in that the receiving phase comprises an immobilised solid phase material supported by a solid support.

Preferably, the receiving phase is adapted to be maintained, in use, in close proximity to the diffusion-limiting membrane such that there is maintained a substantially zero diffusion gap between the two components. In any case, it is preferred that any diffusion gap is less than about 1mm and more preferably is substantially equal only to the thickness of the membrane. The membrane itself may be a separate component from the receiving phase, or may be integral therewith such that it is ordinarily inseparable from the receiving phase and may comprise a layer thereof, whether by surface coating or impregnation, or the like.

Devices for monitoring micropollutants comprising a solid receiving phase are known for monitoring air pollutants, such as the use of activated charcoal in Cao and Hewitt

(Environ. Technol. 12 1055-62 (1991)). Such devices are now commercially available. However, it has not previously been proposed to use such devices in an aquatic environment since, *inter alia*, they would not be capable of withstanding the usual conditions of turbulence and the like pertaining in aquatic environments. Furthermore, they comprise a substantially greater diffusion gap than the devices of the present invention, having about an order of magnitude greater gap between the receiving phase and any membrane.

Accordingly, the present invention further provides a method for monitoring micropollutants in a polluted environment, which method comprises:

(a) providing an immobilised solid receiving phase for the micropollutants;

(b) providing a diffusion-limiting membrane adapted to allow rate-limited diffusion therethrough of the micropollutants and, in use, adapted to separate the receiving phase from the polluted environment;

(c) bringing the membrane into contact with the polluted environment for a sufficient period of time to allow

the pollutants to collect in the immobilised solid receiving phase; and

(d) removing the solid receiving phase from the environment,

characterised in that the environment is an aqueous environment and the solid receiving phase is adapted therefor.

Preferably, the method employed is one wherein the solid receiving phase is a solid phase material supported by (such as on or in) a solid support, such as in the device according to the invention. Preferably, the solid receiving phase is immobilised on a backing plate or support plate positioned against the receiving phase on its face remote from the diffusion-limiting membrane, thereby substantially eliminating any air gap between the receiving phase and the aqueous environment, when in use. This is advantageous as there will be no danger of leakage or loss of receiving phase by means of damage to the device in the aquatic environment.

The device preferably comprises an immobilised non-polar, chromatographic and/or chelating receiving phase

separated from the aqueous environment by means of a diffusion limiting membrane, whereby, in use, dissolved analytes pass through the membrane, across a fixed and substantially constant diffusion gap (effectively the thickness of the membrane) to the hydrophobic chromatographic and/or chelating phase, where they are retained until desorbed.

Accordingly, a preferred method of the invention is wherein the solid receiving phase is removed from the environment and is further removed from the device; eluted with a desorbent for desorbing the micropollutants therefrom; and the eluate analysed. Preferably, the solid receiving phase is a unitary element adapted to be placed directly in a stream of desorbent, from which the desorbent plus micropollutants can be eluted and collected in one step and/or adapted to be placed directly in analytical equipment, such as gas chromatography, atomic absorption, mass spectrometry or laser ablation equipment.

The device is preferably calibrated under a wide variety of conditions for a test-set of analytes with a range of physico-chemical properties. The device and method of the invention are suitable for testing for and monitoring

the presence of both organic analytes and inorganic analytes, including pesticides, agrochemicals, polychlorinated biphenyls, such as those listed in Table 2 of Example A hereinafter; metals, such as copper (*eg* Cu(I)), zinc, cadmium, lead, arsenic, mercury and chromium (*eg* Cr (VI)); and other inorganic elements, including nutrients, such as P (*eg* PO_4) and N (*eg* NH_4, NO_3). Especially preferred is a device or method according to the invention suitable for monitoring organic micropollutants. The final, calibrated device therefore provides a means for the quantitative determination of time-averaged levels of analytes in aquatic environments during extended deployment times. It is robust enough to be placed in a wide range of aquatic environments without adversely affecting its performance and is therefore a significant advance in the field of environmental monitoring.

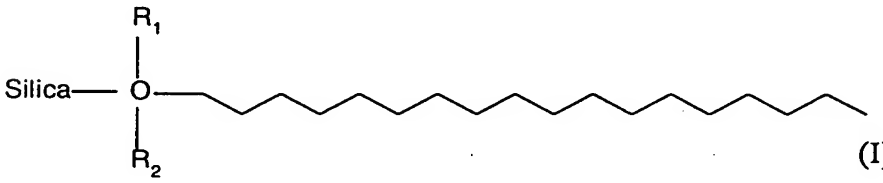
Another advantage of the device according to the present invention is that it is more easily customised in order to be useful for testing a range of pollutants - whether several at one time or specific, single pollutants.

Choice of the material for the solid receiving phase will therefore depend, *inter alia*, on the nature of the

particular micropollutant to be monitored. The process of uptake in passive sampling systems is basically one of equilibrium partitioning. In the case of organic analytes, for example, non-polar micropollutant molecules have a higher affinity for the organic receiving phase than the surrounding aqueous environment and therefore accumulate in the sampler to levels exceeding those found in the environment. Thus the rate of uptake is controlled by the nature of the semi-permeable membrane, but the position of equilibrium and therefore the capacity of the device is governed by the receiving phase.

A range of commercially-available, chromatographic materials with different physico-chemical properties can be used in the device. Some of these solid-phase materials have a proven high affinity to non-polar analytes, for example, having been used for the extraction of organic pollutants from aqueous media. Previous research has also found that non-polar organic compounds stored on hydrophobic, solid-phase materials have increased stability over those stored refrigerated in aqueous samples. Examples of some suitable solid phase materials include hydrophobic chromatographic phases such as organo-silicas (*e.g.* C_6, C_8, C_{18}

hydrocarbon bonded silicas) and polymers (*e.g.* poly(styrenedivinylbenzene)), *eg* those of formulae (I) - (III) below for organic compounds, and chelating (*eg* iminoacetates) and ion-exchange materials for metallic species.



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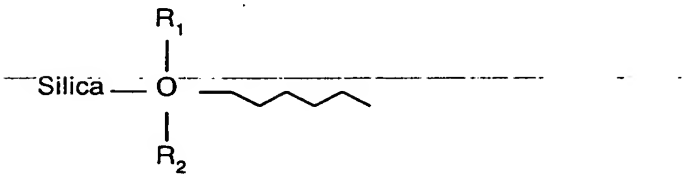

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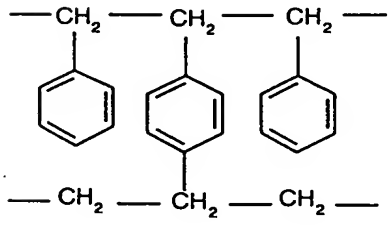


(III)

Further advantages are obtained when the solid, receiving phase is in the form of a cartridge or disk, particularly a disk that is suitable for either transfer from the device to a standard filter funnel for removal of the collected analytes or (in the case of metals) direct laser analysis. In this way, the collected micropollutants on the solid receiving phase can be

conveniently extracted and measured in the normal way. Such forms of the solid receiving phase (*ie* disks, cartridges or otherwise wherein the receiving phase itself is intimately associated with a solid substrate) further afford robustness to the device and prevent dispersal into the environment of the receiving phase in the event that damage occurs to the protecting elements of the device (*eg* the diffusion-limiting membrane). Particularly where the diffusion-limiting membrane is a separate component of substantially the same shape as the receiving phase or is a layer thereof (*eg* coating thereon), then these two components of the device are not able to move relative to each other, thereby further preventing damage to the device or the environment, in use. Such a device is thereby fully submersible in aquatic environments.

Some suitable disks for use as the solid receiving phase in the device of the invention are already commercially available, such as C_8 or C_{18} disks (available from 3M), C_{18} cartridges (such as Varian Bond Elut), and chelating or ion-exchange disks (also from 3M). In some cases, the solid receiving phase may be available in combination with a matrix, such as a PTFE fibril or glass fibre matrix. Examples of these include PTFE-based C_{18} or C_8

disks (3M Empore™) and glass fibre-based C_{18} disks (Supelco Envi-Disk). Particularly useful are C_{18} Empore™ disks, which comprise octadecyl chain length hydrocarbon groups bonded in a silica-based polymer enmeshed in PTFE fibres.

Therefore, a preferred device according to the invention is one wherein the solid receiving phase is immobilised on a solid substrate, such as a matrix, which may then itself be further immobilised against a backing plate or support. In the case where the diffusion-limiting membrane is integral with the receiving phase, then this may also be coated on or impregnated into the substrate.

Accordingly, the method of the invention may further comprise pre-treating the solid receiving phase, such as by coating or impregnating it with the diffusion-limiting membrane; by conditioning it with a conditioner; by treating it with an agent adapted to complex, chelate or otherwise assist the receiving phase to receive and retain the chosen micropollutant, such as a photometric agent; or by loading it with internal standard; or the like; or any combination thereof.

Depending upon the particular solid receiving phase

chosen and the pre-treatment applied to it before use, it is possible for the device to be custom-adapted for monitoring one or more analytes at a time. Hence, the solid receiving phase may comprise a chromatographic and/or chelating phase that has been treated with an agent for complexing or chelating the chosen analyte(s), such as metallic species. For example, if C_{18} or C_8 disks are used in different matrixes (PTFE, glass fibre) together with a photometric reagent (*eg* bathocuproine or 1,5-diphenylcarbohydrazide), it is possible to collect specific metal species (*eg* Cu(I), Cr(VI), respectively). Normally, it is difficult to detect low concentrations of such species with only the photometric reagent bathocuproine, but together with preconcentration of the metal complexed to a photometric reagent on a C_8 or a C_{18} disk, it is possible (as described by Björklund and Morrison *ibid*). The possibility of having the photometric reagent immobilised on the disk in advance or to have it directly equilibrated in the sample is also an advantage. Examples of photometric reagents that can be used are bathocuproine, methylthymol blue, xylenol orange, glycine cresol red, binchinonic acid and 1,5-diphenyl carbohydrazide.

On the other hand, chelating disks may be used to collect

a plurality of different metals simultaneously. Alternatively, multivalent metal ions may be collected by treating the matrix (*eg* chelating resin disk (3M Empore™ Extraction Disk)) with a complexing agent, such as ammonium acetate, so that the receiving phase contains iminoacetate groups that will complex the metal ions.

In some cases, the chosen solid receiving phase (*eg* in disk form) may require conditioning prior to its use in the device. This conditioning has two effects: it rinses the disk with organic solvent to remove any impurities that may have contaminated the disk during manufacture or storage; and, in the case of use for monitoring organic analytes, it solvates the receiving phase, thereby increasing its ability to sequester any organic analytes to which it may be exposed. For this reason, it is preferred not to allow the disk dry out at any stage between conditioning and deployment of the passive sampling device. The conditioning process generally comprises soaking the disk in HPLC grade methanol solvent for from about 5 to about 20 minutes at room temperature. During this time the disk may take on a transluscent appearance. The disk may then be submerged in ultrapure water to remove excess solvent prior to being removed from the water and dried.

Preferably, the chosen receiving phase is loaded prior to use with an internal standard. This internal standard is an isotopically labelled compound and is designed to diffuse during deployment of the device from the receiving phase through the diffusion-limiting membrane and into the aquatic environment at a known and controlled rate. By using disks pre-loaded with an internal standard, it is expected that the time-averaged water concentrations of aqueous micropollutants can be calculated, taking into account alterations in uptake rates due to variations in temperature, turbulence and biofouling effects.

Choice of the material for the diffusion-limiting membrane should be such that the membrane is diffusion-limiting but nevertheless allows the rapid diffusion of analytes through the membrane to provide the system with a rapid response to fluctuating external concentrations. As long as the membrane is truly diffusion-limiting, the rate of uptake will be dependent on environmental concentration rather than on flow rates in the environment.

A range of membrane materials with very different chemical characteristics and physical properties is

available for evaluation with regard to their suitability as diffusion-limiting membranes for selected micropollutants in the passive sampling system. A membrane material is required which allows the rapid diffusion of the micropollutant, *e.g.* small, organic molecules through its pores into the receiving phase of the device. It should also have a low affinity for the micropollutants for which the device is to be calibrated, so that pollutants do not accumulate within the membrane material rather than passing through to the chosen receiving phase. In addition to its diffusional properties, physical robustness and ability to resist bacterial attack should be taken into consideration, as they can be important characteristics of the chosen membrane material in cases where the device is to be deployed in harsh environmental conditions. Examples of suitable membranes for the collection of organic analytes include hydrophilic or hydrophobic polymer materials having a pore size in the range of from about 0.1 mm to 2 mm, such as those listed below in Table 1.

Table 1

Examples membrane materials suitable for use as diffusion-limiting membranes in the (organics) passive sampling device of this invention.

Membrane	Dimensions	Manufacturer	Information
Polycarbonate	47 mm disk, 0.2 µm pore size	Costar	Chemically inert, hydrophilic material. Pore size of fixed diameter.
Polytetrafluoro-ethylene (PTFE)	1.0 µm pore size, supplied as sheet material bonded to polypropylene protective backing	Pall Europe	Chemically inert hydrophobic membrane. Requires wetting with suitable solvent prior to deployment. Weak structured membrane supplied with polypropylene backing to provide physical strength
Polyvinyledene-difluoride (PVDF)	0.2 µm pore size, supplied as sheet material	Pall Europe	Chemically inert hydrophobic membrane. Requires wetting with suitable solvent prior to deployment. Membrane structure sufficiently strong to support itself without protective backing.
Low density polyethylene	Flexible polymer, no fixed structure but low polymer crystallinity forms transient pores with diameter approx. 10 A.	Sainsburys, Fisher scientific	Highly hydrophobic polymeric material. Chemically and biologically resistant. No fixed pore structure. Variety of membrane thicknesses available.
Polysulphone	0.2 µm pore size, supplied in the form of sheet material	Pall Europe	Hydrophilic membrane with fixed pore structure. High chemical and physical resistance.
Cellulose dialysis membrane	1000 MWCO (molecular weight cut-off) dialysis membrane	Millipore	Relatively hydrophilic membrane with fixed pores of universal size. Allows small organic molecules to pass through but forms a barrier to large organic molecules. Low chemical resistance and liable to attack by fouling organisms.

Diffusion-limiting membranes particularly suitable for use with inorganic analytes include cellulose acetate (*eg* 0.45 μm filter available from Sartorius), GF/C (a glass fibre filter available from Waterman), nylon membranes (*eg* available from Sartorius) and dialysis membranes (*eg* available from Spectra/Por).

The diffusion-limiting membrane may also be treated prior to use. For example, to hinder biofouling and to prevent biofouling from becoming a major problem, the surface of the diffusion limiting membrane may be treated with an anti-fouling agent, biocide and/or Nafion coating. Nafion (available from Aldrich, UK) provides mechanical strength, is chemically stable and is permeable (to certain molecules, such as charged species). It has sulphonic acid groups attached to a polymer backbone. Almost all anions are excluded from the surface of the membrane. Nafion is also affected by pH due to its surface charge. Alternatively, Nafion may itself comprise the diffusion-limiting membrane and may form an integral unit with the solid receiving phase, such as by forming a layer thereon.

The solid support for the solid receiving phase may comprise any inert solid material that can maintain

integrity in an aquatic environment for economic periods of time and that can help to prevent damage to the solid receiving phase in use. The support may itself be integral with the receiving phase or with a body encompassing the device, or it may comprise a separate component that can be fitted to the body or otherwise adapted to provide support to the receiving phase. For example, the support preferably comprises a body in which the removable and/or changeable components of the device are held during use. The support may additionally or instead comprise a supporting disk adjacent the receiving phase on its face remote from the diffusion-limiting membrane. Examples of suitable materials for the solid support include high-density polymers, such as high density polypropylene, PTFE or glass fibre.

In the device according to the invention, the diffusion limiting membrane and the solid receiving phase optionally together with the solid support may be in contact as an integral, optionally disposable, unit. One or more, and preferably all three, of these essential components of the device according to this invention are preferably placed, prior to use, in a body adapted to receive it/them. Therefore, the present invention further provides a unit for use as a passive sampling

device, which unit comprises a device according to the invention and an inert body (which may itself comprise the solid support) adapted to allow insertion therein and removal therefrom of the solid receiving phase and adapted to allow access from the aqueous environment of the micropollutants to the membrane.

Optionally, the device may further be provided with further mechanical protection, in particular that adapted to provide mechanical protection for the diffusion-limiting membrane. Accordingly, netting such as a polypropylene net or a mesh such as stainless steel mesh, may be provided on the face of the membrane away from the receiving phase in order to provide a compact sampler without air in between the receiving phase and the diffusion-limiting membrane; to help prevent any mechanical damage that can occur in the field; and to help prevent large solids or sediment from damaging or contaminating the sampler.

Optionally, the device may further comprise a removable sealing plate to hold in and/or further protect the diffusion limiting membrane during transportation and/or storage. The sealing plate may be held in position in the inert body and/or in relation to the solid support by

securing means, such as a screw-threaded ring. The sealing plate may also be adapted to form a cap or stopper to enable water or other conditioning fluid(s) to be maintained in contact with the solid receiving phase, such as to prevent its drying out between preparation and use of the device.

The present invention will now be illustrated by way of example only with reference to the following drawing(s), in which:

Figure 1 is an exploded longitudinal section through a device for organic sampling according to the invention;

Figures 2 and 3 show cross-sectional and perspective views, respectively, of a device for inorganic sampling according to the invention;

Figures 4 and 5 show cross-sectional and perspective views, respectively, of a device having a protective mesh and sealing plate according to the invention;

Figure 1 shows a passive sampling unit comprising a substantially cylindrical PTFE body (1) having at its rear end one lug (2) adapted to provide fastening means

for the unit in use, to prevent it from floating off-site. The body (1) is in two parts - a front part (3) and a rear part (4), which are water-tightly joinable by machined screw thread (5). When undone, the screw thread enables the unit to be taken apart to remove or insert the chromatographic receiving phase disk (6), which is positioned between the diffusion limiting membrane (7) to the front and a thin, rigid supporting PTFE disk (8) to the rear. In this example, the disk (8) has a diameter of 50 mm and is 5 mm thick. Any visible air bubbles are smoothed away from between the membrane (7) and receiving phase disk (6). On the opposite face, the supporting disc (8) has a machined round lug (*e.g.* 10 mm long and 5 mm in diameter) of PTFE with a small aperture (*e.g.* 3 mm in diameter) through which the device can be suspended during laboratory tank studies or field sampling (*e.g.* by wire or nylon cord (not shown)). The loaded supporting disc (8) (with receiving phase (6) and diffusion limiting membrane (7)) may be manually inserted into a locating ring in the front part (3) of the PTFE body (1). Holding the supporting disc (8) in position, the rear part (4) of the PTFE body (1) may be screwed into the front part of the body (1) to form a watertight seal between the outer face of the diffusion limiting membrane and the front segment of the body (1). Thus, the membrane (7) and

supporting disk (8) are held in place between the two halves of the body (1). The front face (3) of the body (1) is open to allow contact between the aqueous environment (and the micropollutants therein) (not shown) and the membrane (7).

A similar embodiment is shown in Figures 2 and 3, but wherein the diffusion-limiting membrane (27) is protected by a 500 μm polypropylene net (29) on its face remote from the receiving phase (26). In this embodiment, the receiving phase (26) itself comprises a 3M Empore™ Extraction Disk; the diffusion-limiting membrane (27) is a Nafion 117-coated cellulose acetate membrane; and the supporting disk (28) is made of polypropylene.

Likewise, Figures 4 and 5 show a similar embodiment to that of Figure 1, but wherein the diffusion-limiting membrane (7) is protected by a stainless steel mesh (9), and the unit body (1) is further provided with a screw-threaded ring (11) for securing a sealing plate (12) for fitting into the base of the unit when not in use in the field.

In order that time-averaged aqueous concentrations can be calculated from accumulation of analytes in a passive

sampling system, it is necessary that the rate of accumulation of compounds in the device is known. Furthermore, the effects on this calibration of fluctuating environmental conditions during the field deployment should be accounted for. For this reason, extensive calibrations of the passive sampling units of Figures 1 to 3 (hereinafter sometimes referred to as "the prototype") have been carried out in a controlled laboratory environment. The effects of analyte concentration, water turbulence, temperature and device orientation on the uptake rates of the different prototypes have been assessed and the results obtained are described in Examples 3 to 6, respectively for the organic prototype, and the effects of time and concentration for the inorganic/chelating prototype in Example 7.

EXAMPLE A

Analysis of Organic Micropollutants - Techniques

Organic Determinands - Test Micropollutants

In order to evaluate the prototype passive sampling system, the behaviour of a test set of six compounds: diuron, atrazine, phenanthrene, PCB 52, dieldrin and PCB 153 was chosen for study. The structures of these compounds are shown below. These compounds were chosen

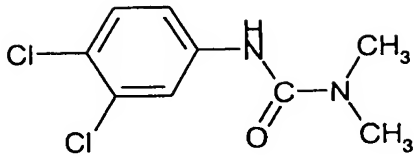
as they are known organic micropollutants commonly found in the aquatic environment and have a wide range of physico-chemical properties (see table 2).

Table 2

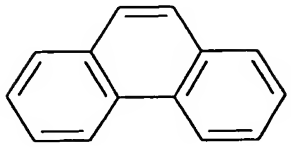
Some physico-chemical properties of the test set of organic compounds included for initial assessment as to their behaviour in the prototype passive sampling systems.

Compound	Molecular mass	Log K_{ow}	Solubility in water
Diuron	248	2.80	0.042 gl^{-1} at 25°C
Atrazine	205	2.50	30 mgl^{-1} at 20°C
Phenanthrene	268	4.46	1.3 mgl^{-1} at 20°C
PCB 52	290	6.26	0.03 mgl^{-1}
Dieldrin	344	4.58	0.186 mgl^{-1} at 25°C
PCB 153	358	7.45	0.00105 mgl^{-1}

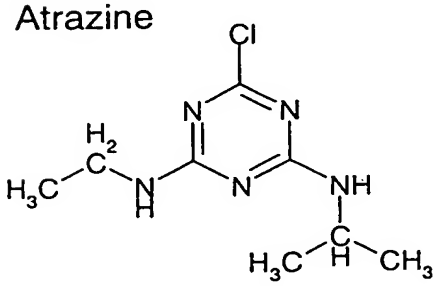
Diuron



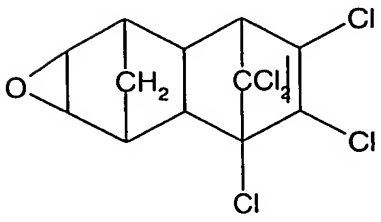
Phenanthrene



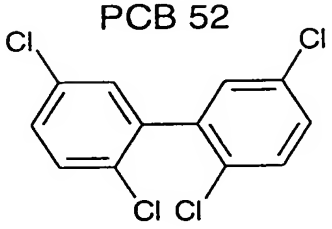
Atrazine



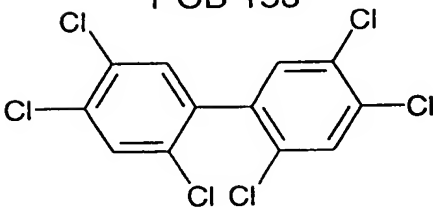
Dieldrin



PCB 52



PCB 153



Techniques have been established for the quantitative isolation of the test set of six compounds both from aqueous and solid-phase media, and their subsequent determination using gas chromatography with mass selective detection.

Quantitative analysis of organic micropollutants by gas chromatography with mass selective detection (GC-MSD).

The six test compounds were simultaneously determined using gas chromatography with mass selective detection. The deuterated internal standard ^{10}D-phenanthrene was used to correct for volumetric inaccuracies during sample preparation.

Materials:

Certified pure reference standards of each organic analyte were purchased from Qm_x Saffron Walden, UK, (purity > 98% in all cases). Individual stocks were produced at 1 mg/ml in HPLC grade acetone. Mixed calibration standards were prepared by suitable dilution of these stock solutions into HPLC grade 2,2,4-trimethylpentane. The gas chromatograph conditions and selective ion monitoring conditions (see table 3) are listed below.

Calibration Range:	0 - 2000 ng/ml
Instrument Conditions:	
Instrument:	Hewlett Packard 5890 GC with autosampler and mass selective detector
Mode:	Selective Ion Monitoring (SIM)
Column:	HP-5 (Crosslinked 5% phenyl methyl silicone) 30 m * 0.25 mm * 0.25 µm film thickness
Carrier gas:	Helium at 1ml/min
Injection mode:	Pulsed splitless injection (2 min splitless)
Injection volume:	2 µl
Injector temperature:	275°C
Detector Temperature:	300°C
GC oven temperatures:	Initial temperature 90°C held for 3.0 minutes ramped at 20°C/min Final temperature 280°C held 3.0 mins

Table 3

Selective ion monitoring conditions used for the determination of the test set of organic determinands by GC-MSD.

Compound	Retention Time (min)	Target Ion (mass units)	Qualifier Ion (mass units)
^{10}D-Phenanthrene (ISTD)	9.54	188	80
Diuron	6.25	187	124
Atrazine	9.31	200	215
Phenanthrene	9.57	178	176
PCB 52	10.38	292	220
Dieldrin	11.56	79	263
PCB153	12.04	360	290

Extraction and pre-concentration of the test set of organic compounds from an aqueous matrix

The six test compounds were extracted from aqueous solution by means of liquid-liquid extraction using two 10 ml aliquots of HPLC grade dichloromethane. 100 μl of a 1 ng/μl solution of ^{10}D-phenanthrene in HPLC grade 2,2,4-trimethylpentane was added to the extract as an internal standard. The solvent was exchanged for 2,2,4-trimethylpentane and the sample concentrated under a stream of nitrogen to a volume of exactly 1.0 ml. The final extract was transferred to a 2 ml autosampler vial in preparation for analysis of pollutant levels by gas chromatography with mass selective detection (GC-MSD; see above).

Procedural blanks and recovery standards

With each batch of samples extracted, a procedural blank consisting of a 100 ml aliquot of ultrapure water and two recovery solutions consisting of 100 ml ultrapure water spiked with 100 ng of each organic compound to be analysed were extracted. The recovery standards were analysed alongside unextracted calibration standards and a mean percentage recovery for the two extracts was calculated. This recovery factor was applied to the results of the samples in the same batch.

Thus sample results were produced, which were compensated for less than 100% extraction efficiency and batch to batch variations in method performance. Procedural blanks were again analysed alongside unextracted calibration standards to check for analytical interferences entering the system during the extraction procedure.

Typical percentage recoveries for each of the compounds contained in the test set are shown in table 4.

Table 4.

Method performance of each organic analyte in the test set extracted from aqueous media and analysed using GC-MSD.

Compound	Mean % recovery	% STD Deviation	Number of replicates
Diuron	104.2	5.6	10
Atrazine	106.7	4.0	10
Phenanthrene	99.2	3.4	10
PCB 52	98.0	4.1	10
Dieldrin	99.7	5.6	10
PCB 153	82.5	5.9	10

EXAMPLE B - Method of Organic Continuous-Flow Studies

An apparatus was developed which allows the constant mixing of organic analytes with water in an exposure tank.

This flow-through system was devised to carry out laboratory evaluations of the prototype device under controlled conditions. Ultrapure water is pumped and, separately, organic determinands dissolved in methanol are injected by syringe into the exposure tank at known and controlled rates. The tank has a fixed volume and an overflow to waste. This allows for the solution contained within the exposure tank to have a controlled (fixed or fluctuating) concentration of organic analytes for the long periods of time required for the laboratory

calibrations of the prototype device. Furthermore, the tank can be stirred at a known rate using an overhead glass stirrer and the whole apparatus is situated in a controlled temperature environment.

Therefore, the rates of diffusion of all the test compounds through the chosen membrane materials and into the chosen receiving phase could be ascertained in a controlled laboratory environment.

Calibration of the prototype device with different diffusion-limiting membrane materials.

The diffusion rates of the test set of organic determinands through differing membrane materials were tested by installing each membrane as the diffusion limiting membrane in the prototype device, and exposing the device to a solution of pesticides in the exposure tank of the flow-through system. In each experiment, a C_{18} Empore™ disk (available from 3M) was used as the chromatographic receiving phase and the whole prototype device was exposed to an aqueous solution of organic analytes at known concentration for a fixed period of 48 h. During this experiment, the stirring speed was set at 140 rpm; the temperature of the system was kept constant

at 11°C; and dissolved analyte concentration was controlled and constant. 100 ml aliquots were taken from the exposure tank daily and the concentration of each analyte in the exposure tank was plotted on a daily basis. The prototype device was removed from the exposure tank following the 48 h exposure period and the mass of each analyte accumulated in both the C_{18} Empore™ disk and the chosen membrane material was determined. This experiment was carried out in triplicate for each membrane material.

48 h accumulation factors (MAF_{48}) were calculated for both the receiving phase and each diffusion-limiting membrane material. This accumulation factor gives a measurement of the uptake rate of the device fitted with different diffusion-limiting membranes related to the concentration of each analyte to which it is exposed and was calculated as follows:

48 h accumulation factor (MAF_{48}) = C_d / C_w

where: C_d = Concentration accumulated in the C_{18} receiving phase following exposure for 48 h in units of ng $disk^{-1}$.

C_w = Average concentration in the exposure tank during deployment in units of ng ml^{-1}.

EXAMPLE C

Materials and Methods used in Inorganic Tests

C_{18} disks and cartridges

The C_{18} cartridge was Varian Bond Elut and the C_{18} disks were 3M Empore™ (PTFE-support) and Supelco Envi-disk (glass fibre-support). Before separation, all cartridges and disks were conditioned with methanol (5 ml) and washed with ultrapure water (2 x 5 ml). Cartridges were held in a Varian vacuum assembly and disks were held in a Millipore filtration assembly.

Equilibration involved addition of hydroxylamine hydrochloride (reducing agent), sodium citrate (buffer adjusted to pH 4.3) and bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline-disulphonic acid, as photometric reagent) to an unfiltered sample. The final concentrations were: hydroxylamine hydrochloride, 0.12 M; sodium citrate, 0.01 M; bathocuproine, 0.036 M. Unless stated otherwise, all water samples contained 5% methanol to prevent loss of cartridge or disk conditioning. Bathocuproine was immobilised on the cartridge or disk by passage of 0.36 M bathocuproine in citrate buffer. Reducing agent, citrate buffer and methanol were added to

the sample before passage over the immobilised bathocuproine. Total metal concentration was determined after ultraviolet irradiation of sample (100 ml), in the presence of 30% hydrogen peroxide (500 µl) and concentrated nitric acid (100 µl Scanpure), in a Metrohm UV-digester.

The Cu(I)-bathocuproine complex was eluted with methanol:water (90:10, vol:vol) from the cartridge (3 x 2 ml) or disk (3 x 5 ml). For each elution, the eluate was allowed to soak by drawing through one drop, standing for two minutes and then filtering through the rest of the eluate. Solutions were made up to volume with methanol:water (90:10, vol:vol) for cartridge (10 ml) or disk (25 ml). Absorbance (484 nm) was measured in a 1 cm cell in a Philips PU8620 spectrophotometer. The detection limit could be improved by the use of a longer cell. Each analysis was accompanied by a sample blank without added bathocuproine reagent. The blank was unreacted reagent and humic substances and was subtracted from the final result. The bathocuproine method for the determination of Cu (I) is described in more detail elsewhere (Moffett, *et al.*, Analytica Chimica Acta 175, 171 (1985)). A linear calibration curve was found for 0-50 mgl^{-1} Cu. Higher

concentrations were not tested. All results for Cu determinations reported were carried out in at least duplicate and were within a relative standard deviation of ± 1%.

Chelating disk

The chelating resin disk (3M Empore™ Extraction Disk) was conditioned with ultrapure water (20 ml), 3M HNO_3, ultrapure water (2 x 40 ml), 0.1 M ammonium acetate pH 5.3 (50 ml) (Riedel-de Haen) and ultrapure water (2 x 40 ml). The chelating disk then contained iminoacetate groups in the matrix of inert PTFE, which collects multivalent metal ions by forming a complex similar to EDTA.

After the sampling period, the metals collected *in situ* on the exposed chelating disk in the passive sampler were either eluted with 3M HNO_3 (3 x 8 ml) and analysed with an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) to get the metal concentration, or the disk was analysed directly without elution, by direct laser ablation ICP-MS (LA-ICP-MS). Samples as 0.24 M HNO_3 were analysed for Cu, Zn, Cd and Pb by (ICP-MS) with correction for molecular ion interferences. The instrument used was a Perkin Elmer

Elan 6000 and the laser ablation system coupled was a CETAC LSX 200.

Net

The net was made from polypropylene (500 μm, available from Industri Textil Job AB), and was acid washed (10% HNO_3) and rinsed with ultrapure water before use.

Prototype Passive sampler

The passive sampler (Figures 2 and 3) was made of polypropylene, and was acid washed (10 % HNO_3) and rinsed before use. After conditioning, the receiving disk (*e.g.* chelating disk, C_{18}) was placed in the passive sampler together with the diffusion limiting membrane and the protective net. The polypropylene holder was finally screwed together to make a water-tight seal. After exposure, the sampler was unscrewed, and the membranes were taken out and analysed.

Biofouling - Nafion coating (charge exclusion-negative charge)

The diffusion-limiting membrane was treated with a Nafion coating (0.05 % w/v, Nafion 117, Aldrich (Poole, UK) in an ethanol solution 1 ml). Periphyton experiments were

done in a small creek Hultabäcken outside Göteborg. Periphyton is the term often used for the kind of microbial community that developes on an artificial substratum such as glass disks. The colonisation of a clean glass surface was initiated by bacteria. Periphyton were established on round, 14 mm diameter glass discs by using the periphyton sampling method of Blanck *et al*, in ASTM STP 988 American Society for Testing and Materials, Philadelphia, 219-230 (1998). The glass disks were held vertically by polyethylene holders attached to a polyethylene frame. The frame was attached to the bottom of the sampled stream by a polyethylene pole and left *in situ* for 2 to 3 weeks. The disks were impregnated with different amounts of Nafion to find out if Nafion could hinder biological growth. Growth was found to be slower with Nafion. The disks were analysed for chlorophyll a, the indicator of algal biomass. Five periphyton glass disks were handled together. They were cleaned on all sides, but the rough surface side, put into 1 ml of DMSO (dimethyl sulphoxide) and stored frozen until analysed. All handling and analysis were done in dim light. Then the chlorophyll a was extracted at 60°C over 30 min. Once the samples had cooled down, an equal amount of acetone was added and the sample was centrifuged. The sample was then analysed spectrophotometrically. The chlorophyll a

concentration was calculated according to the equation given by Jeffrey and Humphrey, Biochem. Physiol. Planzen. 167, 191-194 (1975).

LA-ICP-MS

Laser ablation studies were done on chelating disks prepared with different metal concentrations in the laboratory and on disks from passive samplers that had been out in the field. A laser ablation system was coupled to the ICP-MS for the laser ablation studies and used to analyse chelating disks directly (see Figure 6, which shows direct Cu calibration of chelating disks by rastering the surface with an ultraviolet laser and direct injection of the Ar flow into the ICP-MS. The average intensity (counts), used for calibration, is obtained from the plateaux. In this case, the elution step of the disk was excluded and a raster, series of three lines ('rastering'), was run on the surface, although it is also possible to go deeper in the matrix.

Laser ablation furthers the *in situ* advantages of passive sampling by direct analysis of the sampling chelating surface without sample preparation. It thereby avoids the contamination and sample alteration risks encountered during filtration and preparation of bottle samples.

Preparation and use of the passive sampler and method according to this invention will now be illustrated by the following examples.

EXAMPLE 1

Use of Prototype Passive Sampling Device having C_{18} Disks

Preparation of C_{18} Empore™ disks

C_{18} Empore™ disks required conditioning prior to their use as receiving phase for the passive sampling system. The conditioning process consisted of soaking the disks in HPLC grade methanol (10 ml, from Fischer Scientific) for 20 minutes in a glass beaker. During this time the disk took on a transluscent appearance. The disks were then transferred to a glass beaker or petri dish containing ultrapure water to remove excess methanol. The disk was removed for the water and pressed gently with a clean, dry tissue to remove excess water.

Disks of membrane material were prepared with a diameter of 47mm. The conditioned C_{18} disks were laid directly onto the PTFE loading or support disk.

The diffusion-limiting membrane was placed over the C_{18}

disk and any visible air bubbles were smoothed away from between the two membranes. The loading disk (complete with chromatographic phase and diffusion-limiting membrane) was then placed into the device body (Figure 1) and screwed in place to form a watertight seal.

Storage and deployment of the passive sampling device

The C_{18} disks were not allowed to dry out between conditioning and deployment of the device. Therefore, either the devices were loaded immediately before deployment or the loaded devices were stored immersed in ultrapure water in a sealed vessel, such as a glass jar, prior to deployment. They should be transported stored in this way to the deployment site and removed only immediately prior to use. Alternatively, the passive sampler can be filled with distilled water and sealed using a sealing plate, as in the case of the sampler shown in Figures 4 and 5, component (12) until deployment. The device was removed from the storage jar and a support wire or cord was fastened to the lug on the PTFE support disc. The wire or cord was of sufficient length to allow the device, once deployed, to be suspended totally submerged beneath the water surface throughout the sampling period. In the field, the wire or cord may be secured to a buoy or similar structure.

The device was lowered into the water and any air trapped in the front face of the sampler body was removed by briefly inverting the assembly. The device was then allowed to hang freely in the water. The device naturally hangs with the exposed face of the diffusion limiting membrane pointing downwards.

Retrieval

After the required deployment period, the device was taken from the sampling site and disassembled. Disassembly followed the reverse of that described above for assembly. The chromatographic receiving phase was removed, such as by using clean stainless steel forceps, and was placed in a clean glass screw top vial fitted with a PTFE-lined cap. The chromatographic phase was stored at -20°C prior to analysis. The diffusion limiting membrane was discarded. The PTFE body and supporting disc were washed with water and organic solvents prior to re-use.

Extraction of the test set of organic compounds from C_{18} Empore disks

The organic determinands were extracted from the C_{18} Empore™ disks using 5 ml of HPLC grade acetone (for 5 min in an ultrasonic bath) followed by 10 ml of 50%:50%

(vol/vol) ethylacetate:2,2,4-trimethylpentane mixture (for 5 min in an ultrasonic bath). At the end of this time, the disk was removed, the two solvent extracts were combined and 100 µl of the 1 ng/µl solution of deuterated ^{10}D-phenanthrene added as an internal standard. The extract was concentrated to a volume of 1 ml under a stream of nitrogen and the extract was placed into 2 ml auto-sampler vials in preparation for determination by GC-MSD (see Example B).

EXAMPLE 2
Evaluation of membrane materials suitable for use in the prototype

The diffusional properties of some commercially available membrane materials (see Table 1) were assessed by exposure of the prototype sampler of Example 1 in the continuous-flow system as described in Example B. The accumulation of organic analytes in the C_{18} receiving phase following 48 h exposure of the prototype device fitted with each membrane material is summarised in Table 5. The experiment was carried out at constant analyte concentration, at a temperature of 11°C and at a constant stirring speed of 140 rpm. Error bars show standard deviations (n = 3). Results are quoted as an accumulation factor (ml/device) = concentration in the

receiving phase (ng/device) / mean concentration of the analyte in the aqueous phase during deployment (ng/ml). Numbers in parentheses show standard deviations (n=3).

Table 5.

	Diuron	Atrazine	Phenanthrene	PCB 52	Dieldrin	PCB 153
No membrane	844 (268)	962 (59)	1164 (128)	1118 (115)	1094 (42)	1048 (145)
0.2 µm pore size polysulphone membrane	140 (12)	168 (17)	0 (0)	0 (0)	0 (0)	0 (0)
1.0 µm pore size PTFE membrane	84 (4)	98 (3)	101 (7)	16 (9)	52 (5)	53 (24)
0.2 µm pore size polycarbonate membrane	150 (8)	148 (8)	11.7 (5)	7.1 (6)	13 (14)	5 (38)
0.2 µm pore size PVDF membrane	115 (7)	106 (9)	22 (3)	18 (8)	19 (2)	11 (3)
glass fibre	51 (21)	52 (20)	118 (10)	81 (6)	67 (10)	43 (8)
1000 MWCO cellulose dialysis membrane	150 (30)	121 (16)	143 (9)	20 (13)	94 (23)	7 (29)
Polyethylene (type 1)	0 (0)	0 (0)	1028 (125)	740 (240)	256 (55)	233 (115)
Polyethylene (type 2)	0 (0)	0 (0)	1048 (95)	819 (81)	114 (14)	215 (40)
Polyethylene (type 3)	0 (0)	0 (0)	643 (16)	262 (15)	1.0 (1)	68 (11)
PVC	0 (0)	0 (0)	276 (84)	94 (30)	19 (11)	17 (6)

The pattern of accumulation of compounds in the C_{18} receiving phase is affected by the physico-chemical properties of both the membrane material and the target

analyte. Some of the more non-polar compounds such as the PCBs, dieldrin and phenanthrene have a high affinity for a particular membrane material and therefore accumulate within the structure of this membrane and do not pass through into the receiving phase. In contrast, some of the polymeric membranes provided for rapid transport of non-polar analytes but formed a barrier for the transport of the more polar analytes such as diuron and atrazine.

The pattern of accumulation of organic analytes within the two compartments of the passive sampling system fitted with different membrane materials is illustrated in Table 6. The device is fitted withwith a C_{18} Empore™ disk as the receiving phase and either a 0.2mm pore size polysulphone membrane or a polyethylene membrane as the diffusion limiting membrane material. Accumulation was measured following a 48 hour exposure to aqueous solution of analytes at constant concentration, constant stirring speed (140 rpm) and constant temperature (11°C). Results quoted are the average of three replicates and are expressed as an accumulation factor (ml/device) = concentration in the tested compartment (ng/device) / mean concentration of the analyte in the aqueous phase during deployment (ng/ml); values in parentheses show standard deviations (n=3).

Table 6

	Diuron	Atrazine	Phenanthrene	PCB 52	Dieldrin	PCB 153
C_{18} Empore™ disk with polysulphone membrane	140 (12)	168 (17)	0 (0)	0 (0)	0 (0)	0 (0)
Polysulphone membrane	64 (21)	9 (8)	935 (188)	875 (169)	634 (107)	498 171)
C_{18} Empore™ disk with polyethylene membrane	0 (0)	0 (0)	1028 (125)	740 (240)	256 (55)	233 (115)
Polyethylene membrane	0 (0)	0 (0)	48 (4)	116 (47)	143 (15)	177 (141)

In particular, can be noted:

The polysulphone membrane has a high degree of physical strength and is available in a low protein binding formula that may have antifouling properties. It has a high affinity for the more hydrophobic compounds included in this study *i.e.* phenanthrene, dieldrin and the PCBs, but the more polar compounds such as atrazine and diuron readily pass through this membrane material and are

accumulated in the receiving phase of the passive sampling device. The polysulphone membrane is therefore suitable for use in a passive sampling system for the targeting of relatively polar micropollutants with typical log K_{ow} values of less than 4.

The polyethylene membrane allows the rapid diffusion of non-polar analytes, but forms a barrier for the diffusion of relatively polar compounds with log K_{ow} < 4, such as atrazine and diuron. This material is therefore a suitable diffusion-limiting membrane for a passive sampling system targeting non-polar organic micropollutants.

The following section and Examples 3 to 5 describe the results obtained from laboratory exposures of the prototype device in controlled conditions in the flow-through system described in Example B. Accumulation of analytes within the receiving phase of the passive sampling system following exposure was determined by means of solvent extraction and gas chromatographic analysis as described in Example A.

In each experiment, the prototype device was tested fitted with both the polyethylene and the polysulphone membrane. Therefore the results quoted for the non-polar analytes

phenanthrene, dieldrin, PCB 52 and PCB 153 were produced using the system fitted with the polyethylene membrane and the results quoted for the more polar analytes diuron and atrazine were produced using the polysulphone system.

Calibration of the prototype passive sampling systems at constant exposure concentration over time

Table 7 shows the uptake pattern of organic analytes by the passive sampling device exposed in a controlled laboratory environment to a constant concentration of organic analytes for a range of different exposure periods. Accumulation of organic analytes by a passive sampling system fitted with a C_{18} Empore™ disk as receiving phase and either a 0.2 μm pore size polysulphone membrane or a polyethylene membrane as a diffusion-limiting membrane material. Accumulation was measured following exposure of the system to an aqueous solution of analytes at constant concentration, stirring speed (140 rpm) and temperature (11°C). Linear regressions of the accumulation factor, AF, (ml/device) on time were plotted and are given with the coefficients of variation (R^2) in table 7. AF = concentration of analytes in the tested compartment (ng/device) / mean concentration of the analyte in the aqueous phase during deployment (ng/ml).

Table 7

Analyte	Linear regression	R^2 value
Diuron*	Y = 1.64x + 13.7	0.954
Atrazine*	Y = 2.42x + 4.15	0.990
Phenanthrene°	Y = 25.51x + 131.9	0.939
PCB 52°	Y = 29.55x + 149.5	0.984
Dieldrin°	Y = 9.16x – 100.6	0.973
PCB 153°	Y = 13.40x – 182.8	0.909

* sampler fitted with polysulphone membrane, ° sampler fitted with polyethylene membrane.

The pattern of uptake in each case is linear with time over deployment periods ranging from several hours to 9 days. R^2 values in each case are above 0.9. Linear regressions plotted through the data points show the lag time between exposure of the device to an aqueous solution of each analyte and its uptake into the receiving phase of the passive sampling system. In effect this is a measurement of the response time of a particular prototype passive sampling system to a particular compound. In the case of the uptake of phenanthrene and PCB 52 into the polyethylene device, only a small lag phase is evident between exposure to these analytes dissolved in an aqueous environment and uptake into the C_{18} receiving phase. In the case of the uptake of PCB 153 and dieldrin, on the

other hand, a more significant lag phase is observed between exposure to dissolved compounds in aqueous solution and uptake into the passive sampler.

As each graph is essentially linear, the time-averaged concentration of each of the dissolved analytes in aqueous solution can be easily predicted from the final analyte concentration in the receiving phase of the passive sampling system at the end of a deployment period.

EXAMPLE 3

Effect of exposure concentration on the uptake of organic analytes

According to the principles of Fickian diffusion, the uptake of each organic analyte by the receiving phase of the passive sampling system should be linearly related to the analyte concentration in the surrounding water. The device with chosen diffusion-limiting membrane and C_{18} Empore disk as receiving phase was placed into the exposure tank according to Example B containing a known concentration of dissolved organic analytes at a temperature of 11°C and with the exposure tank stirred at 140 rpm. The device was exposed to this fixed concentration for a period of 48 h following which it was

removed and the mass of each analyte accumulated in the receiving phase of the device was measured (see Example 1 and Example A - GC-MSD). 100 ml samples were taken from the exposure tank daily and the concentration of each analyte in the exposure tank was plotted on a daily basis. This experiment was repeated at a range of dissolved analytes concentrations.

Table 8 shows accumulation of each organic compound in the receiving phase of the prototype passive sampling device following exposure to different analyte concentrations in the controlled flow-through system for 48 h period. Linear regressions were effected for the accumualtion factor of each analyte, AF, (ml/device) on the mean water concentration during deployment (ng/ml) and the resulting equations are given with the coefficients of determination (R^2) in table 8. AF = analyte concentration in the tested compartment (ng/device) / mean concentration of the analyte in the aqueous phase during deployment (ng/ml).

Table 8

Analyte	Linear regression	R^2 value
Diuron*	Y = 0.0871x + 5.12	0.997
Atrazine*	Y = 0.1231x + 5.95	0.994
Phenanthrene·	Y = 1.126x + 28.7	0.974
PCB 52·	Y = 1.024x - 4.01	0.951
Dieldrin·	Y = 0.409x - 9.84	0.985
PCB 153·	Y = 0.673x + 1.48	0.969

* sampler fitted with polysulphone membrane, • sampler fitted with polyethylene membrane.

Again, the calibration graphs plotted are essentially linear, with R^2 values ranging from 0.9510 in the case of PCB 52 to 0.9969 in the case of diuron. This shows that the rate of uptake of each analyte into the passive sampling device is dependent on concentration in the aqueous environment. The mass of organic analytes accumulated in the receiving phase of the passive sampler following a known deployment period is therefore directly related to the concentration to which the device has been exposed.

EXAMPLE 4

Effect of temperature on uptake rate of organic analytes

The prototype device was placed in the exposure tank of Example B calibrated at fixed analyte concentration and a stirring speed of 140 rpm for time periods ranging from one day to one week. The initial experiment was undertaken in a controlled temperature environment with a resultant water temperature of 11°C. At the end of each exposure the prototype device was removed and the mass of each analyte accumulated within the receiving phase was determined. The experiment was repeated in its entirety at temperatures of 4°C, 7°C and 15°C to assess the effect of temperature on uptake rates of organic analytes by the passive sampling system.

Table 9 shows the results; the natural logarithm of the slope was regressed upon the reciprocal of absolute temperature to yield the regression coefficients (slopes in table 9). These slopes have units of K and when multiplied by the universal gas constant ($JK^{-1}mol^{-1}$) give activation energies ($Jmol^{-1}$). The coefficients of determination (R^2) give a measure of the magnitude of the association of the natural log of the rate and 1/T in each case.

Table 9

Analyte	Linear regression	R^2 value
Diuron*	Y = -5083x + 18.75	0.778
Atrazine*	Y = -4464x + 16.67	0.938
Phenanthrene·	Y = -3228x + 14.45	0.876
PCB 52·	Y = -3099x + 14.06	0.717
Dieldrin·	Y = -9153x + 34.19	0.940
PCB 153·	Y = -3855x + 16.10	0.602

* sampler fitted with polysulphone membrane, • sampler fitted with polyethylene membrane.

Alteration in exposure temperature produces an effect on the uptake pattern of organic analytes by the passive sampling system. This effect is largest in the case of the uptake of dieldrin by the device fitted with a polyethylene diffusion-limiting membrane.

EXAMPLE 5

Effect of device orientation on the uptake rates of organic analytes

The prototype device was calibrated by placing in the exposure tank of the flow-through system of Example B for a period of 48 h. The exposure concentration was kept constant, the temperature was set at 11°C and the system was stirred at 140 rpm during this experiment. At the end

of each exposure the prototype device was removed and the mass of each analyte accumulated within the receiving phase was determined. This experiment was carried out with five replicates with the diffusion limiting membrane in a vertical orientation and a horizontal orientation to investigate the effect of device orientation on the uptake rate of organic analytes by the passive sampling device.

48 hour exposure of the prototype passive sampling device with the membrane in a vertical orientation and horizontal orientation showed that the orientation of the device has no significant effect on uptake rates of the organic analytes by the passive sampling systems. This is the case with both the polysulphone device and the polyethylene device.

Example 6 relates to a passive sampler (inorganic prototype, Figures 2 and 3) according to the invention comprising a 3M Empore™ Extraction Disk (chelating disk) as the receiving disk; a Nafion 117-coated cellulose acetate diffusion-limiting membrane and a 500 mm polypropylene net, as described in Example C.

EXAMPLE 6

Laboratory Tests using Chelating Prototype

Experiments were done to calibrate the device. Time-exposed passive sampler experiments were done in a Cu and Zn concentration of 500 µg/l, in 300 ml H_2O and during 1 - 5 hours with magnetic stirring. The passive samplers were taken out of the water hourly during 5 hours and were then analysed for Cu and Zn in a flame atomic absorption spectrometer, AAS (Perkin Elmer 2380). Calibration lines for Cu of $y = 78.182x + 104.07$, $R^2 = 0.9796$, for Zn of $y = 94.554x + 99.655$, $R^2 = 0.9789$, respectively, were found, where y is the concentration in µg/l and x is the time in hours.

Experiments were done in the same way for concentration-exposed passive samplers from 50 - 500 µg/l for Cu and Zn, in 300 ml H_2O and during 5 hours with magnetic stirring. All the passive samplers were taken out of the water after 5 hours and analyzed. The calibration line for Cu was $y = 0.0427x + 1.1333$, $R^2 = 0.9937$, and for Zn was $y = 0.2117x + 29.689$, $R^2 = 0.9864$, where x is the concentration in µg/l and y is the absorbance in %.

EXAMPLE 7

Use of Prototype Passive Sampling Device having Internal Standard

The method of Example 1 was followed, with the following adaptations: first, the conditioned C_{18} disks were preloaded with an internal standard. The internal standard was introduced to compensate for alterations to analyte uptake by the passive sampler due to fluctuations in environmental conditions (*eg* temperature and turbulence) during deployment. The conditioned C_{18} Empore™ disk was placed in a standard filtration apparatus. 100 ml of ultrapure water were spiked with 1000 μl of a solution of 100 ng/μl d8-naphthalene and d6-dimethylphthalate in methanol. The solution was filtered through the C_{18} Empore™ disk under vacuum and the disk was immediately placed into the device with the diffusion-limiting membrane.

Secondly, the device was that of Figures 4 and 5. The device was placed face up, and a few millilitres of ultrapure water were placed in the upper cavity before the system was sealed for transportation and storage using the sealing plate.

During deployment, the sealing plate was removed from the device, and a stainless steel protective mesh was fitted in place and secured using the screw thread ring provided.

After the required deployment period, the device was taken from the sampling site and the inner cavity of the device was filled with a few milliliters of water from the sampling site. The sealing plate was screwed tightly onto the device to create a watertight seal. For field use, the device should be refrigerated (or preferably frozen at -20°C) for transportation prior to analysis. The system was disassembled (reversing the procedure outlined above for assembly) immediately prior to analysis, as described in Example 1.

Following deployment, the mass of analyte accumulated within the passive sampler was found to be proportional to the mass of internal standard lost form the sampler for a range of temperatures and levels of water turbulence. Therefore, rate of accumulation of analyte could be adjusted for differing environmental conditions.

FIG. 1

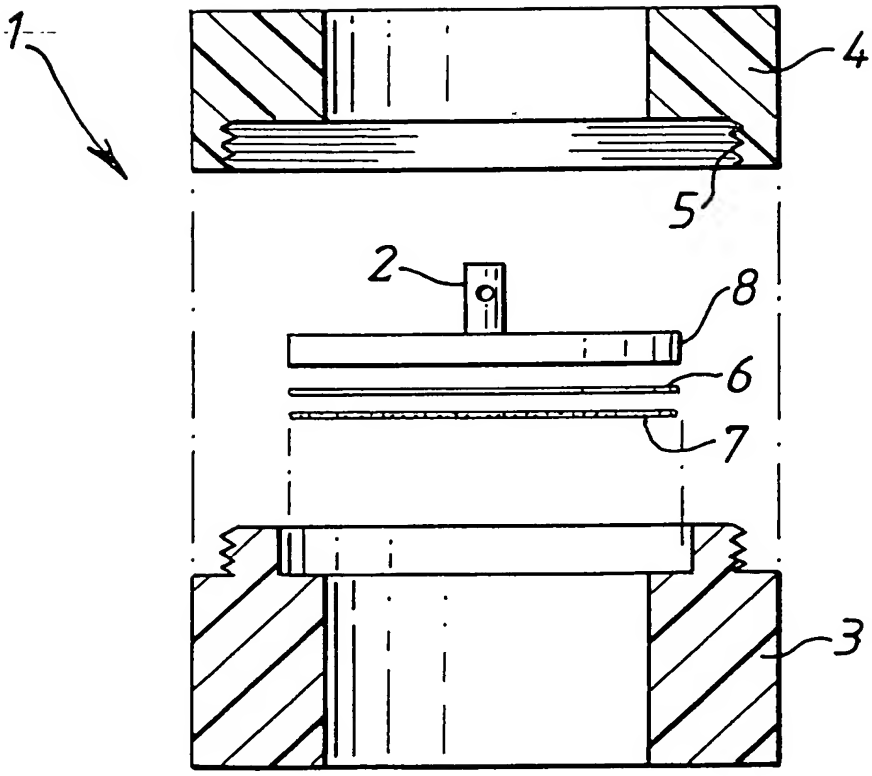


FIG. 2

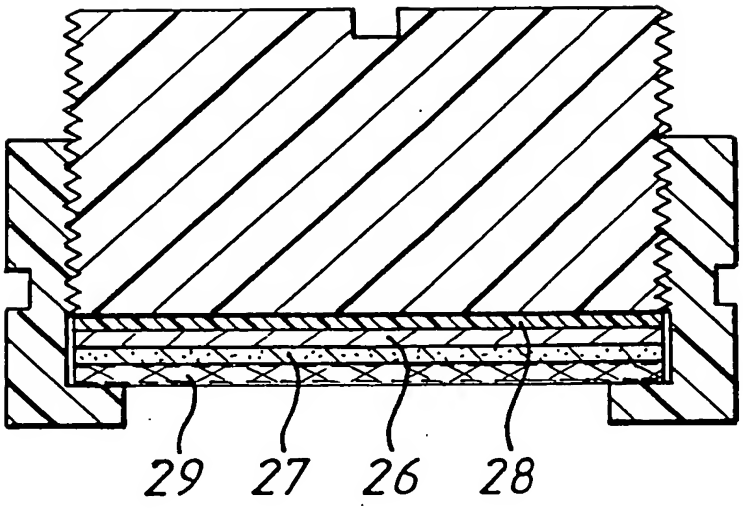


FIG. 3

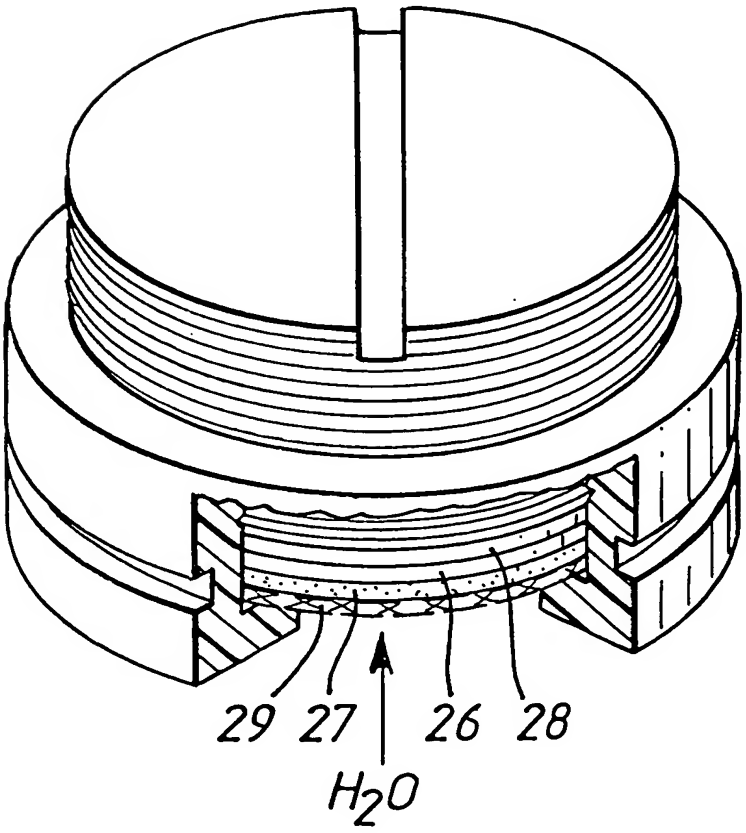


FIG. 4

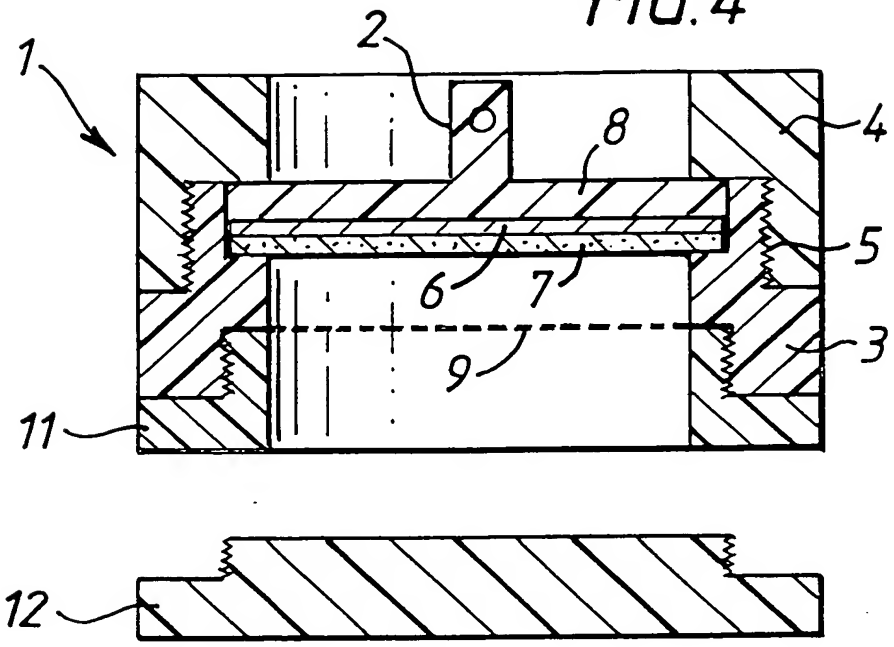


FIG. 5

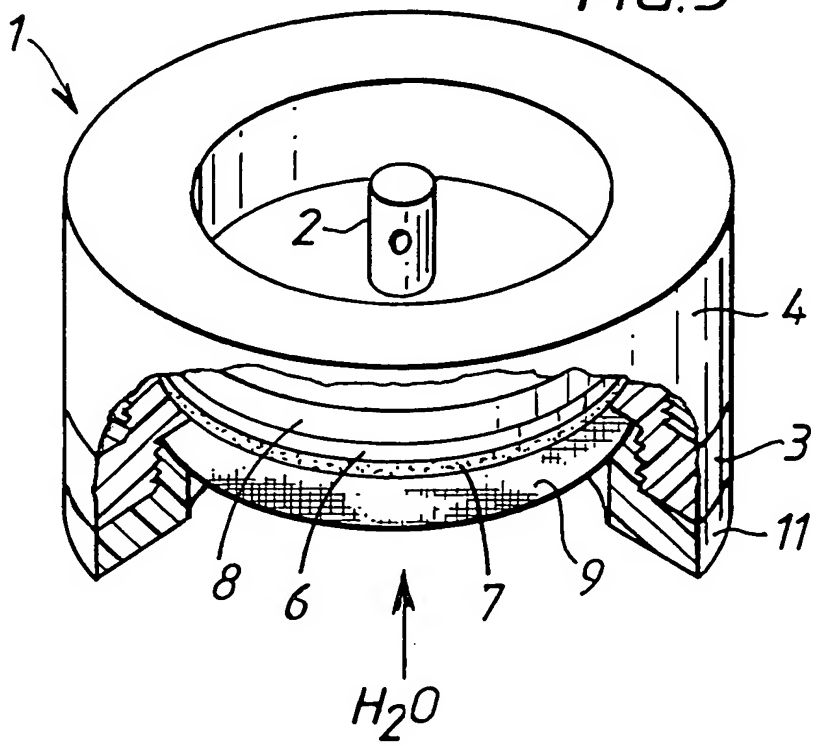
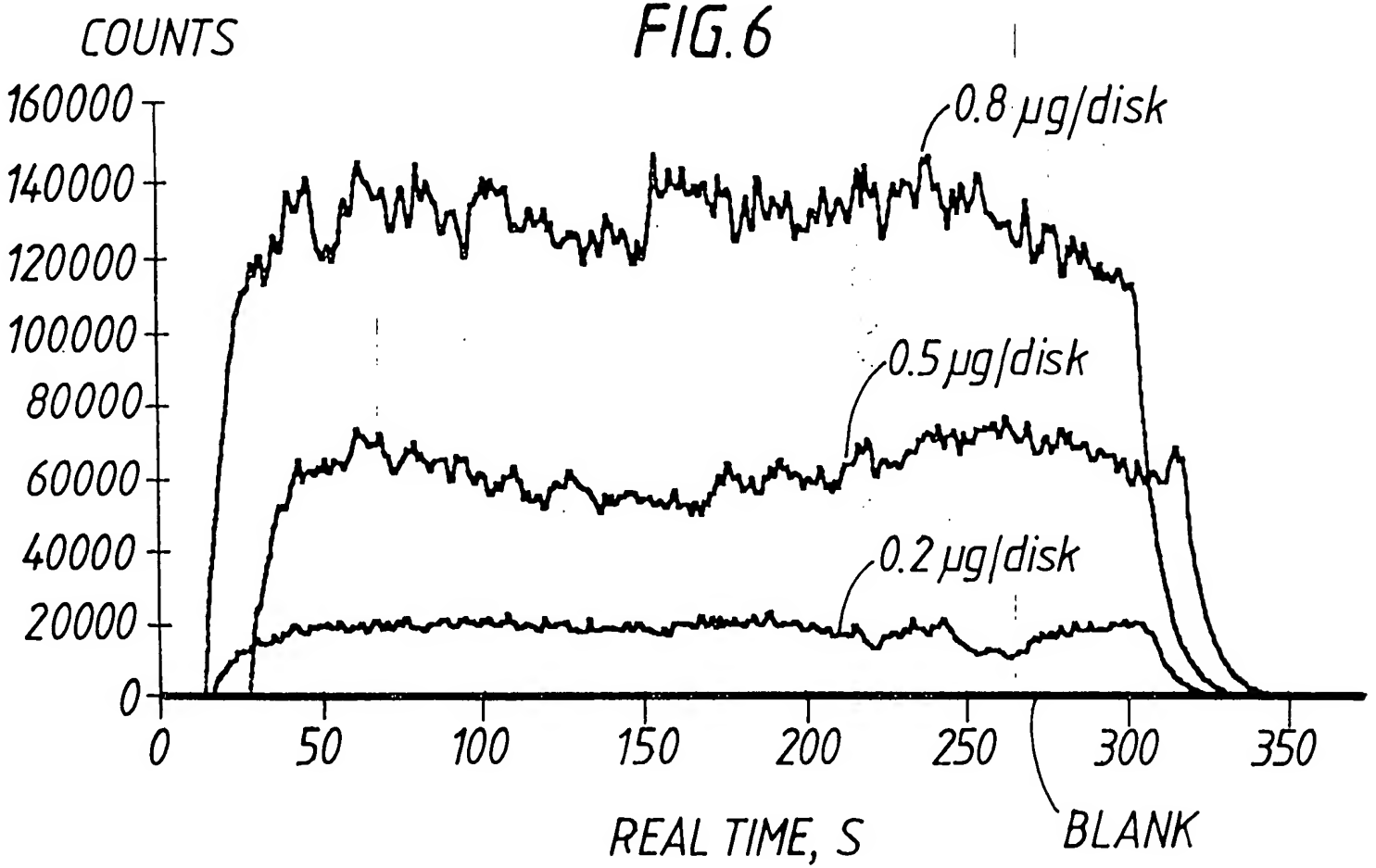


FIG.6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60000
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0.5 µg/disk
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0
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200
250
300
350
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